Sustained Release of Bevacizumab Towards Improving the Treatment of Age-Related Macular Degeneration

Eman Mirdamadi[1,2], Sangyoon Kim[1,2] and Tao L. Lowe[1,2]

[1]Fischell Department of Bioengineering, A. James Clark School of Engineering, University of Maryland, College Park, MD. [2]Department of Oral and Maxillofacial Surgery, School of Dentistry, University of Maryland, Baltimore, MD

Statement of Purpose: Age related macular degeneration (AMD) is the leading cause of blindness in people aged over 65 and is projected to affect 288 million people globally by 2040 [1,2]. An estimate global cost of visual impairment due to AMD is about $343 billion [2]. A form of AMD, known as wet AMD, is a chronic eye disorder that occurs when abnormally growing and fenestrated blood vessels enter the macula, causing sudden or progressive blindness. Bevacizumab (Avastin®, Genentech) is an anti-vascular endothelial growth factor (VEGF) monoclonal antibody therapy which is the gold standard treatment for wet AMD [3]. However, current bevacizumab regimens require monthly intravitreal injections which cause complications such as elevated intraocular pressure and patient noncompliance [4]. Current efforts addressing this challenge involve encapsulating bevacizumab in nanoparticles, hydrogels and implants. However, these delivery vehicles currently have limited *in vivo* effect, no more than 2 weeks of *in vitro* bevacizumab release, use organic solvents that denature bevacizumab, and require difficult surgical interventions that can cause further eye damage [5]. In this work, we aim to develop a thermo-responsive and biodegradable nanogel system that can load bevacizumab in aqueous solutions which maintain bevacizumab integrity, sustain the release of intact bevacizumab for several months *in vitro*, and reduce angiogenesis and vascular leakage *in vivo* for the treatment of AMD.

Methods: A UV emulsion polymerization method is used to synthesize nanogels composed of thermoresponsive poly(N-isopropylacrylamide) and biodegradable dextran-poly(lactate-2-hydroxyethyl-methacrylate) with and without 4-15 wt% bevacizumab in aqueous solution. The synthesized nanogels are purified by dialysis and dried by lyophilization. The particle size and size distribution of the nanogels are measured using dynamic light scattering (DLS, Nanobrook Omni). Bevacizumab release from the nanogels at 20 mg/mL in PBS (pH 7.4) are studied using Spectra/Por® Float-A-Lyzer G2 dialysis devices (300K MWCO, Spectrum® Laboratories). The release media are collected daily during the first week, twice per week through day 28, and then weekly afterwards. The amounts of bevacizumab in the release media are quantified using UV absorbance at 280 nm on a UPLC system (Waters Acquity UPLC® H-Class and Acquity BEH C18 Column). The 24 h cytotoxicity of the nanogels at concentrations of 0, 0.1, 0.2, 0.5, 1, 2, 5, and 10 mg/mL to fetal human retinal pigment epithelial (fhRPE) cells at a seeding density of 50000 cells/cm^2 in 96-well plates were assessed using a MTT assay.

Results: The DLS results show that the hydrodynamic size of the nanogels at 37 °C increase 27% from 115.04 ± 10.80 nm to 146.07 ± 10.60 nm after bevacizumab is encapsulated in the nanogels. The nanogels sustain bevacizumab release in PBS (pH 7.4) for at least one week with zero order release kinetics (Fig. 1). The nanogels are not cytotoxic to fhRPE cells with above 90% cell viability at concentrations up to 10 mg/mL after 24 h of incubation (Fig. 2).

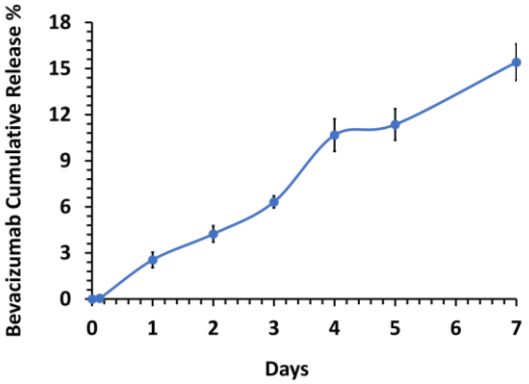

Figure 1. Cumulative release of bevacizumab from our nanogels as a function of time (n = 4).

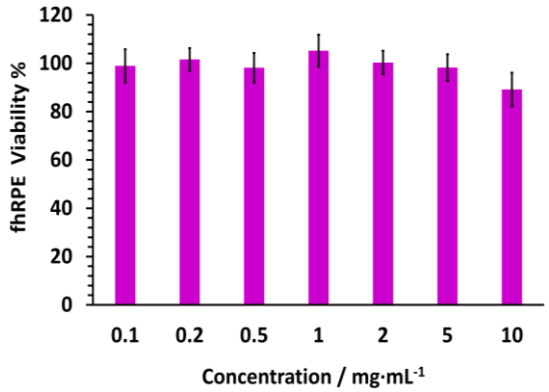

Figure 2. Cytotoxicity of non-bevacizumab loaded nanogels to fhRPE cells at a series of concentrations after 24 h of incubation (n = 4).

Conclusion: We have developed a non-toxic nanogel system that can load bevacizumab in aqueous environment and sustain the release of bevacizumab in PBS (pH 7.4) for at least one week. Immediate future work will continue to characterize the chemical and physical properties as well as release profiles of bevacizumab-loaded nanogels for over 3 months. Completion of this project will have a significant impact on the treatment for AMD with better efficacy and patient compliance.

References:

[1] CDC. https://www.cdc.gov/visionhealth/resources/features" https://www.cdc.gov/visionhealth/resources/features /macular-degeneration.html.
[2] Retia International. https://retina-international.org/retina-action-call-to-action-launch/
[3] Villegas VM. Expert Opin Drug Deliv. 2017;14:273-282.
[4] Falavarjani K. Eye. 2013;27:787-794.
[5] Seah I. Eye, 2020;34(8):1341-1356.